THE PHYSIOLOGY OF FLOWERING, WITH ESPECIAL REFERENCE TO FLORAL INITIATION IN SOYBEANS

BY

ARTHUR WILLIAM GALSTON

B. S., Cornell University, 1940

M. S., University of Illinois, 1942

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN BOTANY IN THE GRADUATE SCHOOL OF THE UNIVERSITY OF ILLINOIS, 1943

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Introduction

Since 1920, when Garner and Allard discovered photoperiodism, it has been known that reproductive activities in many species of plants may be initiated by exposure of the vegetative parts to appropriate combinations of light and dark periods. However, the internal biochemical changes preceding flowering have remained an almost complete mystery.

Many investigators have attempted to elucidate these internal causes by chemical analysis of vegetative and reproductive plants, correlating differences in composition or functions with the change in behavior of the plant. Unfortunately, much of this work is invalid, since flowering itself is known to *result* in widespread metabolic alterations. In order for a chemical change to be assigned a causal role in flowering, it must be demonstrated that the change occurs prior to the morphological differentiation of floral primordia, and also that experimental production of such a change within the plant consistently leads to a flowering condition.

Detailed examination of the literature reveals widespread disagreement among various workers as to the internal events preceding flowering. Whereas an early theory emphasized quantitative relationships of carbon and nitrogen fractions in the plant, more recent work indicates that the changed C:N ratio at the time of flower appearance bears no causal relation to floral initiation, and may even be a result of the flowering process. Some experimenters have claimed a rise, others a fall, and still others no change in catalase activity at the time of floral initiation. The claim has been made that the photosynthetic rate rises at floral initiation, and also that it falls at this time. Respiration is said to rise at flowering time, and also to fall. The cambium is said to become more active, and also less active. Much evidence in-

dicates that transpiration rise and tissue desiccation occur at floral initiation, but contrary evidence may also be found. The data on vitamins, pigments and mineral patterns are too scanty to be judged critically.

This lack of agreement throws serious doubt upon the generally made assumption that all plants starting to flower have widespread biochemical characteristics in common. Recent evidence pointing to a hormonal control of flowering tends to indicate that the only characteristic common to all such plants is the production and accumulation of a floral hormone.

In 1936, several workers independently obtained evidence for the presence in plants of a mobile floral stimulus, provisionally named *florigen*. Evidence for its existence is based mainly on transmission of the stimulus between parts of the same plant, and between graft partners. Its passage, in grafts, among different varieties, species, genera and photoperiodic types, indicates that florigen is the same in all plants. This hormonal theory is reinforced by the fact that minute amounts of several synthetic compounds are known to cause floral initiation. Complete acceptance of the theory is impossible, however, until successful extraction of the hormone has been accomplished.

The present work aims: (a) to review the literature dealing with the causes of floral initiation; (b) to repeat certain critical experiments bearing on the florigen theory; (c) to investigate the relation of the already known plant hormones to floral initiation, (d) to attempt extraction and characterization of the floral stimulus, and (e) to attempt to establish a physiological criterion for the onset of flowering which might be of use in developing a bioassay for florigen.

Experimental

A. Repetition of critical experiments on production and translocation of the floral stimulus.

Previous studies have indicated that several varieties of soybeans show different photoperiodic behavior. Such photoperiodic diversity within so closely related a group of plants rec-

ommended the use of soybeans as the experimental material in these studies.

Experiment 1—The photoperiodic behavior of four soybean varieties.

Pots of Agate, Batorawka, Biloxi and Peking soybeans were exposed to various photoperiods. The former two varieties were found to be "indifferent" photoperiodically, whereas the latter two are of the "short-day" type and will not flower when exposed to photoperiods of 17 hours or longer.

Experiment 2—Photoinduction of the short-day varieties.

Peking and Biloxi soybeans grown for one month under a 17-hour photoperiod were given seven consecutive 8-hour days, then returned to long-day conditions. Examination of buds with a hand lens thirty days after the completion of the short-day treatment revealed abundant floral primordia on almost all of the plants so treated.

Experiment 3—Transmission of the floral stimulus by leaf grafts.

Agate, Batorawka, Biloxi and Peking soybeans were grown continuously under a 17-hour day. Using a previously described technique for the grafting of single leaves, Agate and Batorawka donors were grafted onto Peking and Biloxi receptors. In all successful grafts, the single donor leaf was sufficient to induce flowering over the entire receptor within one month. The control grafts of Peking leaves on Peking receptors remained vegetative. This experiment indicates the passage of a floral stimulus from Agate and Batorawka leaves to the receptor plant.

Experiment 4—The path of translocation of the floral stimulus.

Experiment 3 was repeated, but the petioles of one-half of the donor leaves were scalded over a small area prior to grafting. The floral stimulus was unable to pass from these leaves, corroborating the view that living cells are necessary for its translocation.

B. ATTEMPTED EXTRACTION AND CHARACTERIZATION OF THE FLORAL HORMONE.

Experiment 1—Attempted extraction from Agate and Batorawka leaves.

Petiolar diffusiates and aqueous extracts of freshly-ground and of dried Agate and Batorawka leaves were made. These extracts were evaporated down to small volume, mixed thoroughly with molten agar, drawn up into cellophane drinking straws and allowed to solidify. Application of such straw segments to petiolar stumps of 17-hour Peking plants failed to induce flowering in these receptors.

Experiment 2—Attempted transmission of the floral stimulus through an aqueous medium.

Receptor cuttings of 17-hour Peking plants were placed with their lower ends in vials containing Hoagland's solution. Donor Agate and 8-hour Peking cuttings were also placed in these vials. After one week, the receptors were rooted in a sand bed, then transplanted to soil in pots. One month later, they showed no evidences of floral initiation.

Experiment 3—Repetition of Moshkov's water-gap experiment.

Moshkov has claimed the transmission of the floral stimulus from a leaf to a receptor across a small water gap. In an attempt to repeat this experiment, month-old 17-hour Peking receptors were defoliated except for one apical leaf. The petiolar stump of the next leaf below was encased in a glass tube fastened to it by a split cork. A similar arrangement at the upper end of the tube made possible the insertion of a donor Agate leaf. Leaves and water were renewed at frequent intervals for eight days. Microscopic examination of the plants one month later revealed no floral initiation.

Experiment 4—Effect of water-soluble growth factors on floral initiation.

Cholodny has suggested that the B vitamins and other plant growth factors may play a part in florigen action. To investigate this hypothesis, solutions of thiamin hydrochloride, p-aminobenzoic acid, biotin concentrate, nicotinic acid, calcium pantothenate, riboflavin, ascorbic acid and indole-3-acetic acid were injected into the flaccid cotyledons of month-old 17-hour Peking receptor plants. After 30 days the plants had produced no floral primordia, indicating that these substances, in themselves, possess no flower-initiating properties.

C. THE RELATION OF AUXINS TO FLOWERING.

Since auxins are known to possess some morphogenetic action, it seemed wise to investigate their possible relation to floral initiation. In these experiments, auxin assays were made by the conventional *Avena* curvature test, the auxin being extracted from the plant materials by peroxide-free ethyl ether.

Experiment 1—The auxin content of Agate and Peking soybeans.

Agate and Peking soybeans were grown under a 17-hour photoperiod. Prior to the appearance of floral primordia on the Agate plants, leaves and apical buds of both varieties were assayed for auxin. The leaves of the two varieties showed no significant differences in auxin level, but Agate tips were distinctly lower in auxin than Peking tips. This suggests that low auxin levels may precede or accompany floral initiation, although the difference may also be varietal, and independent of photoperiodic effects.

Experiment 2—The auxin content of 8-hour and 17-hour Peking plants.

Peking plants were grown under a 17-hour photoperiod for one month. At this time, one group received four short photoperiods, another group received eleven short photoperiods, and a final group continued on the long photoperiod. The results of auxin analyses of leaves and apical buds of these plants indicate that no great changes in auxin content occur during floral initiation. However, after floral initiation is complete and the flower buds are developing (second group), the auxin content rises somewhat.

Experiment 3—The action of 2,3,5-triiodobenzoic acid.

Zimmerman and Hitchcock have shown that 2,3,5-triiodobenzoic acid (TIBA) will cause floral initiation in normally vegetative tomato buds. In the present experiments with uninduced 17-hour Peking soybeans, it was found that soil applications or spraying of the plants with TIBA failed to produce floral initiation. This treatment did produce epinasty of young leaves, shortening of the internodes near the apex, loss of apical dominance, darkening of young buds, and abscission of many buds.

Many of these symptoms could be due to a lowering of auxin levels in the plant.

TIBA sprayed onto plants photoinduced for four days caused a tenfold increase in the number of floral buds produced on the plants. TIBA, when present in great molar excess, was also found to inhibit the action of heteroauxin in the *Avena* curvature test. This dual action suggests further that a decreased auxin level favors floral initiation.

Experiment 4—The action of δ-phenylbutyric acid

δ-phenylbutyric acid, known to be an auxin inhibitor, was found to have no effect on floral initiation in photoinduced or in uninduced Peking soybeans. Thus, florigen must have other effects besides destruction of auxin, since auxin destruction in itself cannot cause floral initiation.

Experiment 5—Effect of heteroauxin injection on floral development.

Heteroauxin was administered by intracotyledonary injection to month-old Peking soybeans which had been photoinduced for six days. The time of appearance of macroscopic flower buds was hastened by ten days over the controls. This suggests that high auxin levels favor development of already formed flower buds. Repetition of this treatment on plants photoinduced for two weeks failed to hasten floral development, probably indicating that auxin levels in these plants were already sufficiently high to give an optimum rate of flower-bud development.

D. THE TRANSPIRATION RATES OF FLOWERING AND VEGETATIVE SOYBEANS.

It would be desirable to have a reliable physiological criterion for the onset of flowering, since this would permit earlier determination of floral initiation, and would presumably detect smaller quantities of florigen in extracts. Because of numerous reports of transpiration increase and water loss of tissues during floral initiation, it was decided to investigate this as a possible criterion.

Month-old vegetative Peking plants growing in crocks were divided into two groups, one receiving seven short-days, the other continuing on long-day. Measurements of the daily water loss of the two groups revealed no clear differences in transpiration rate or trend. There was also no difference in the percentage of water in the leaves or apical buds of the two groups.

Discussion and Conclusions

As a result of the present work and of evidence in the literature, the author feels it possible to conclude that the onset of flowering is governed by a floral hormone produced in the leaf under appropriate photoperiodic conditions. This hormone, which is apparently the same in all plants, is translocated through living cells, may pass a graft union and can move up and down a stem equally well. No successful attempts at its extraction have been recorded. Complete acceptance of the florigen theory is impossible until such extraction has been accomplished.

The bulk of the available evidence indicates that auxin levels in growing points play an important role in flower formation, low auxin levels favoring floral initiation in induced plants, and high auxin levels favoring rapid development of existing floral primordia. Among this evidence is inhibition of stem growth, diminished cambial activity and loss of apical dominance at the time of the floral initiation, analytical data on auxin levels in vegetative and flowering plants, data on the effect of injected auxins, sensitivity of auxins to light, and the dual action of TIBA in favoring floral initiation and in destroying auxin. The suggestion is made that florigen operates through several effects, one of which is the destruction of auxin.

VITA

Arthur William Galston was born on April 21, 1920 in Brooklyn, New York. He attended elementary school in Philadelphia and New York City, then went to James Madison High School in Brooklyn. He was graduated in January 1936.

He entered Cornell University in September 1936, intending to study veterinary medicine, but was attracted to botany instead. He was awarded a University Undergraduate Scholarship and a State Regents Scholarship and was also the recipient, in his senior year, of prizes for debating and for criticism of biological literature. He was graduated in June 1940, receiving the B.S. degree.

In June 1941, he was married to Dale Judith Kuntz of New York City.

From September 1940 until September 1942 he was a teaching Assistant in Botany at the University of Illinois, receiving his M.S. in February 1942. During the summer of 1941, he was Research Assistant at the Cold Spring Harbor Station of the Carnegie Institute of Washington. Since September 1942, he has been a Fellow in Botany at the University of Illinois.

He is a member of Phi Beta Kappa, Phi Kappa Phi, Sigma Xi, Botanical Society of America, Illinois State Academy of Science, and American Association of Scientific Workers.

He is author of:

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